EXPERIMENTS UNDERTAKEN

FOR THE PURPOSE OF RECONCILING SOME OF THE DISCORDANT OBSERVATIONS UPON THE

GLYCOGENIC FUNCTION OF THE LIVER.

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When it was announced by Bernard, in 1848, that he had discovered a new and important function of the liver, there being in this organ a constant production of the same variety of sugar that had long been recognized in the urine of diabetic patients, the great physiological and pathological importance of the discovery, attested, as it was, by experiments which seemed to be absolutely conclusive in their results, excited the most profound scientific interest. During the present century, indeed, there have been few physiological questions which have attracted so much attention; and the observations of Bernard were soon repeated, modified, and extended by experimentalists in different parts of the world. In 1857, Bernard discovered a sugar-forming material in the liver, analogous in its composition and properties to starch; and this seemed to complete the history of glycogenesis.

I do not propose at this time to give an extended review of the experiments which have been made in different parts of the world with the view either of confirming or overthrowing the theory advanced by Bernard; but will discuss the two opinions which are now most prevalent in English and French physiological literature. These two opinions are the following:

Those who accept the experiments of Bernard as conclusive assume that the substance of the liver and the blood in the hepatic veins always contain sugar. This sugar is believed to be formed in the so-called hepatic cells, from the glycogenic matter contained in them; and to be taken up by the blood as it passes through the liver, existing in the hepatic veins, the ascending vena cava, and the right side of the heart. It usually disappears from the blood in its passage through the lungs. Sugar is believed always to exist in the liver, the blood of the hepatic veins, and the right side of the heart, independently of the kind of food used. In the carnivora, the blood of the portal system never contains sugar when the animal is confined to a diet of nitrogenized and fatty matters; but sugar is found none the less invariably in the liver and the vascular system between this organ and the heart.

Others have accepted the view advanced by Dr. Pavy, of Guy's Hospital, who professes to have demonstrated that neither the liver nor the blood circulating between the liver and the heart ever contains sugar during life, but that the sugar which has been found in these situations is the result of a *post-mortem* change of the glycogenic matter, or, as it is called by Dr. Pavy, the amyloid matter of the liver.

These two opposite views are supported by experiments which seem to be conclusive; yet it is evident that, if the observations in both instances be entirely accurate, they must prove precisely the same fact. It was in the hope of harmonizing these discordant opinions, that I undertook some modifications of the experiments of Bernard and Pavy. I will not discuss the accuracy of the methods employed by these eminent observers, but intend merely to follow out a train of reasoning, which seems to me to be fully sustained by experiment, and which I believe will lead to a correct interpretation of the apparently opposite results heretofore attained.

Since the summer of 1858, I have been in the habit of repeating, several times each year, the experiments by which Bernard demonstrated the glycogenic function of the liver, performing the vivisections chiefly as class-demonstrations. I have followed most of the modifications of these experiments, which have been published by Bernard from time to time, and have almost always confirmed in every particular the results obtained by this eminent physiologist. I have never failed to demonstrate the absence of sugar in the blood of the portal system, when the specimens were taken with proper precautions from carnivorous animals that had taken neither starch nor sugar into the alimentary canal. I have found it important to apply a ligature rapidly to the portal vein as it penetrates the liver, and to make a very small opening into the abdominal cavity in this step of the experiment. When I have detected a trace of sugar in the clear extract from the portal blood of an animal in the condition just mentioned, it has been consequent upon delay in seizing the vein; and I have anticipated the probability of

finding sugar from blood, which, under these circumstances, regurgitates from the liver. The necessity of employing these precautions is fully insisted upon by Bernard. I have never failed to find sugar in the blood of the hepatic veins of healthy dogs that had taken neither starch nor sugar into the alimentary canal. In my earlier experiments, I never failed to find a great abundance of sugar in the substance of the liver, in dogs under the same conditions. In one instance, however, in the winter of 1859–'60, I failed to find sugar in the liver of a dog that was affected with what is known as "mange;" but considered this to be due to the peculiar condition of the animal.

On several occasions I have repeated Bernard's experiment of analyzing, for sugar, the portal blood, the substance of the liver, the hepatic blood, the blood from the right side of the heart, the substance of the lungs, the blood from the arterial system, and the substance of the muscles, the kidneys, and the spleen, all the specimens being taken from the same animal. I have always found that sugar existed only in the substance of the liver, the blood from the hepatic veins, and the right side of the heart, and in no other situations; showing, apparently, that sugar is constantly being produced by the liver, and is carried by the circulating blood to the lungs, there to be destroyed. Upon several occasions I have drawn the blood from the right side of the heart of a living animal, by catheterization through an opening into the right external jugular vein—a manipulation which presents no difficulty—and have never failed to find sugar. This experiment I have done without the administration of ether, following the operative procedure described by Bernard.

I have also frequently repeated the experiment of passing a stream of water through the liver from the portal vein, by which all the sugar can be removed in a short time, and testing the substance of the liver a few hours after, it having been kept in the mean time at a temperature of from 80° to 100° Fahrenheit. In this experiment I have always found an abundance of sugar. The glycogenic matter out of which this secondary formation of sugar is supposed to take place, I have extracted and studied after the method proposed by Bernard, and have confirmed his observations on this substance in every particular.

In these experiments I have used the various copper tests—viz., Trommer's, Barreswill's and Fehling's, and have made my clear extracts, generally, by boiling with an excess of sulphate of soda, but very often by mixing the blood or the watery extracts of the tissues with animal charcoal and filtering.

The theory advanced by Pavy, that sugar is not produced by the liver during life, and that, when this substance is found in the liver, it is the result of *post-mortem* change of the glycogenic matter (which he calls the amyloid substance), always seemed to me to be invalidated by the experiment of catheterization of the right side of the heart in a living animal, without the administration of ether; for, in the blood taken under these conditions, the presence of sugar is unmistakable. It being admitted that sugar is contained in the blood passing out of the liver, when ether has been administered, and the fact that sugar is sometimes produced in the body, in cases of diabetes mellitus (for there are undoubted cases in which sugar is discharged in the urine, when neither starch nor sugar has been taken as food), point to the probable nor-

mal production and destruction of this principle in the economy. Sugar can hardly be regarded as a heterologous substance, nor as a product of decomposition; and it constitutes an important article of food, from the fact that it is consumed in the body in connection with certain of the processes of nutrition. The hypothesis, that all the sugar which may be taken as food, and all that results of the digestion of amylaceous matters, is deposited in the liver in the form of amyloid matter, is inadmissible; and the pathology of diabetes cannot be satisfactorily explained by assuming that, under this condition, there is a deficiency in the formation of amyloid substance, by the liver, out of the starchy and saccharine alimentary principles.

Dr. Pavy, however, asserts that the liver never contains sugar during life, but that, after death, it is formed out of the amyloid substance, and its proportion goes on increasing for a number of hours, particularly when the organ is kept at about the temperature of the body. The experiments of Bernard with a liver washed out with a stream of water also show that sugar may be produced after death.

I was led to perform the following experiments, from the fact that, of late years, the experiments in which I have been in the habit of demonstrating the glycogenic function of the liver have inclined me to the opinion that the observations detailed by Dr. Pavy are entirely accurate, and that the error consists in his interpretation of the facts. The circumstances which lead to this view were as follows:

I formerly was in the habit of making my demonstrations of the formation of sugar in the liver upon animals that had been etherized; and then I always obtained a brilliant precipitate from a clear extract of

the substance of the liver, boiled with the test-liquid. I performed the experiment in this way before I had acquired sufficient dexterity to seize the portal vein readily, and to go through with the necessary manipulations with rapidity. I subsequently made the operation by first suddenly breaking up the medulla oblongata, then making a small incision into the abdominal cavity, and seizing the portal vein instantly, and following out the remaining steps of the experiment without delay. In this way, although I always found sugar in the blood of the hepatic veins, I frequently failed to obtain a distinct reaction in the extract of the liver; and the more accurately and rapidly the operation was performed, the more difficult was it to detect sugar in the hepatic substance.

It occurred to me, in reflecting upon these facts, that, inasmuch as no one has assumed that the actual quantity of sugar produced by the liver is very considerable, and as a large quantity of blood (in which the sugar is very soluble) is constantly passing through the organ, precisely as we pass water through its vessels to wash out the sugar, the sugar might be washed out by the blood as fast as it is formed; and really the liver might never contain sugar in its substance, as a physiological condition, although it is constantly engaged in its production. We know that the characteristic elements of the various secretions proper are produced in the substance of the glands, and are washed out at the proper time by liquid derived from the blood, which circulates in their substance during their functional activity in very much greater quantity than during the intervals of secretion. Now, the liver-sugar may be regarded as an element of secretion; and, possibly, it may be completely washed out

of the liver, as fast as it is formed, by the current of blood; the hepatic vein, in this regard, serving as an excretory duct.

To put this hypothesis to the test of experiment, it was necessary to obtain and analyze the liver in a condition as near as possible to that under which it exists in the living organism; and, in carrying out this idea, I made the following experiments:

EXPERIMENT I.—A medium-sized dog, full grown, in good condition, not in digestion, was held upon the operating-table by two assistants, and the abdomen was widely opened by a single sweep of the knife. A portion of the liver, weighing about two ounces, was then cut off and immediately cut into small pieces, which were allowed to fall into boiling water. The time from the first incision until the liver was in the boiling water was twenty-eight seconds. An excess of crystallized sulphate of soda was then added, and the mixture was boiled for about five minutes. It was then thrown upon a filter, and the clear fluid which passed through was tested for sugar by Trommer's test. The reaction was doubtful and presented no marked evidence of sugar.

EXPERIMENT II.—A medium-sized dog, in the same condition as the animal in the first experiment, was held upon the table and a portion of the liver excised as above described. The whole operation occupied twenty-two seconds. But ten seconds elapsed from the time the portion of the liver was cut off until it was in the boiling water. It was boiled for about fifteen minutes, made into a paste with animal charcoal, and thrown upon a filter. The clear fluid which passed through was tested for sugar by Trommer's test. There was no marked evidence of sugar.

EXPERIMENT III.—A large dog, full grown, and fed regularly every day, but not in digestion at the time of the experiment, was held firmly upon the table. This dog had been in the laboratory about a week, and was in a perfectly normal condition. The abdominal cavity was opened, and a piece of the liver cut off and thrown into boiling water, the time occupied in the process being ten seconds. Before the liver was cut up into the boiling water, the blood was rinsed off in cold water. The liver was boiled for about seventeen minutes, mixed with animal charcoal, and the whole thrown upon a filter.

Immediately after cutting off a portion of the liver and throwing it into boiling water, the medulla oblongata was broken up; a ligature was applied to the ascending vena cava just above the renal veins; the chest was opened, and a ligature applied to the vena cava just above the opening of the hepatic veins. A specimen of blood was then taken from the hepatic veins. This portion of the operation occupied not more than one minute. A little water was added to the blood, which was boiled briskly, mixed with animal charcoal, and thrown upon a filter. The liquid which passed through from both specimens was perfectly clear.

While the filtration was going on, Fehling's test liquid (a mixture of sulphate of copper, neutral tartrate of potash, and caustic soda) was made up, so as to be perfectly fresh.

The two liquids were then carefully tested for sugar with this preparation. The extract of the liver presented not the slightest trace of sugar. The extract from the blood of the hepatic veins presented a well-marked deposit of the oxide of copper, revealing unequivocally the presence of a small quantity of sugar.

In these experiments I did not attempt to show the absence of sugar in the blood of the portal system; for it would have been difficult, if not impossible, to have demonstrated this, and at the same time to have obtained the specimens of liver as rapidly as I desired. The fact, that the portal blood in a carnivorous animal, that has taken no saccharine or starchy matters into the alimentary canal, contains no sugar, I regarded as settled by the experiments of Bernard, which I have repeatedly confirmed. Neither did I attempt to show that sugar exists in the liver when a certain period has elapsed after death; for this fact has been demonstrated by all who have experimented on the subject. I only desired to ascertain whether the liver taken from a living animal, and the change of the glycogenic matter arrested before any sugar has had time to make its appearance—if its formation be *post mortem*—really contained sugar. A few seconds only elapsed before the liver was cut up into boiling water (which will effectually arrest the transformation of the glycogenic matter), and the presence of sugar in the decolorized extract could not be demonstrated. In Experiment III., particularly, very delicate tests were employed with the greatest care; and, although the extract of the liver contained no sugar, the presence of sugar in the blood coming from the liver was unmistakable. This experiment was peculiarly successful; and I could hardly expect to be able to collect the specimens with less delay. Anæsthetics were not employed in any of the experiments, and there seemed to be no circumstance which could interfere with the normal character of the specimens examined. The animals were perfectly quiet when the experiments were commenced, and were operated upon as soon as they were put upon the

table, the respiration and circulation being apparently normal.

CONCLUSIONS.

Although these experiments are not entirely new, my interpretation of them serves to harmonize, in my own mind at least, the results obtained by Bernard and by Pavy:

1. A substance exists in the healthy liver, which is capable of being converted into sugar; and inasmuch as this is formed into sugar during life, the sugar being washed away by the blood passing through the liver, it is perfectly proper to call it glycogenic, or sugar-forming, matter.

2. The liver has a glycogenic function, which consists in the constant formation of sugar out of the glycogenic matter, this sugar being carried away by the blood of the hepatic veins, which always contain a certain proportion of sugar, and subserving some purpose in the economy connected with nutrition, as yet imperfectly understood. This production of sugar takes place in the carnivora as well as in those animals that take sugar and starch as food; and is essentially independent of the kind of food taken.

3. During life, the liver contains only the glycogenic matter, and no sugar, because the great mass of blood which is constantly passing through this organ washes out the sugar as fast as it is formed; but after death, or when the circulation is interfered with, the transformation of glycogenic matter into sugar goes on; the sugar is not removed under these conditions, and can then be detected in the substance of the liver.